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Population dynamics of recombinant cultures in a dimensionless time domain

Debashis Roy, Pinaki Bhattacharya *

Department of Chemical Engineering, Jadavpur University, Calcutta 700 032, India

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Abstract

We describe an analysis of the population dynamics of recombinant cultures using a dimensionless time variable. In situations where information on the history or growth dynamics of the culture is desired directly in terms of generations, a dimensionless time domain analysis is preferred to the conventional real time analysis. The dimensionless time domain approach that has been developed in this study appears to be quite effective in obtaining such information. The usefulness of this approach in analysis of practical situations has also been illustrated. This approach is also expected to be particularly useful in investigating the growth dynamics of recombinant cell systems where the culture concentration is a function of the cell histories quantitated in terms of generations (e.g. cultures growing under auxotrophic selection pressure). © 1997 Elsevier Science S.A.

Keywords: Population dynamics; Recombinant cultures; Dimensionless time domain

1. Introduction

Generation specific information on population dynamics of mixed culture systems, especially recombinant cell systems, is difficult, if not impossible, to obtain in a straightforward manner from real time analysis of the system dynamics. Situations might arise in a kinetic study of recombinant cell systems when one is interested in obtaining segregated information on the "history" of the culture or in describing the growth dynamics of the system in terms of generations instead of real time units (hours, days etc.). Such situations call for an analysis of the population dynamics using a dimensionless time variable as described in this communication, instead of the conventional real time analysis. The following sections describe this alternative approach to real time analysis, viz. dimensionless time domain analysis, in the context of recombinant culture dynamics.

2. Analysis

Considering a batch culture (non-recombinant) that is not growing synchronously (in synchronous growth the culture concentration does not change continuously with time but doubles only at discrete instants due to "synchronised" division of all the cells in the culture), the culture concentration X, may be expressed as a function of real time "t", by the differential rate equation:

$$dX/dt = \mu X \tag{1}$$

where the parameter μ is defined as the specific growth rate. If the initial condition is expressed as $X = X_0$ at $t = 0$, then the solution of Eq. (1) takes the form

$$X/X_0 = \exp\left(\int_0^t \mu \, dt\right) \tag{2}$$

During the "exponential growth phase", μ is constant and the right hand side of Eq. (2) may be evaluated directly, i.e.

$$X/X_0 = \exp(\mu t) \tag{3}$$

Let $t = t_d$ denote the time required for the culture concentration to double, i.e. $(X/X_0) = 2$ at $t = t_d$. Substituting these values into Eq. (3) one obtains an expression for the doubling time, t_d:

$$t_d = (\ln 2)/\mu \tag{4}$$

For an exponentially growing culture, μ is constant, thus it follows from Eq. (4), that the culture doubling time, t_d is constant too. Now, substituting for μ from Eq. (4) in Eq. (3), the relation

* Corresponding author.

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$$(X/X_0) = 2^{t/t_d} \tag{5}$$

is obtained. Thus, if $0 < t/t_d < 1$, then the culture is in the first generation, if $1 < t/t_d < 2$, the culture is in the second generation and so on. Eq. (5) may, therefore, be looked upon as a restatement of the exponential growth law to the base 2 with the term (t/t_d) denoting the number of generations—integral or fractional. In order to obtain a relation analogous to Eq. (5) for the general case of growth (when μ is not constant), a variable, τ, (say), may be defined by the relation

$$\tau = \left(\int_0^t \mu \, dt\right) \Big/ \ln 2 \tag{6}$$

which may then be used to restate Eq. (2) in terms of τ:

$$X/X_0 = 2^{\tau} \tag{7}$$

Since μ has dimensions of $(\text{time})^{-1}$, it is evident from Eq. (6) that the variable τ is dimensionless. Again, since the term (t/t_d) is dimensionless, it appears from a comparison of Eqs. (5) and (7) that τ indeed represents a dimensionless time. In fact, Eq. (7) implies that at the instant τ (in the dimensionless time domain) the culture concentration has increased 2^{τ} times with respect to its value at the initial instant (i.e. $\tau = 0$). Thus τ-domain analysis (e.g. Eq. (7)) may be considered as an alternative to real time, i.e. t-domain analysis in situations where direct information on the history or growth behaviour of the culture, in terms of generations, is desired. If $I[\tau]$ denotes the integral part of τ, then, "for a culture in the jth generation", it follows that

$$(j-1) = I[\tau] \tag{8}$$

The extension of these concepts to recombinant cell cultures may now be undertaken. The real time dynamics of recombinant cultures may be described by the rate equations developed originally by Imanaka and Aiba [1]:

$$dX^+/dt = (1-p)\mu^+ X^+ \tag{9}$$

$$dX^-/dt = p\mu^+ X^+ + \mu^- X^- \tag{10}$$

where superscripts + and − denote plasmid-bearing (P^+) and plasmidless (P^-) strains respectively and "p" is the relative segregation rate. Let us first consider the dynamics of the P^+ population. If $p > 0$, signifying that P^- cells may arise out of the division of P^+ cells due to plasmid segregation, then the doubling time t_d^+ is distinctly different from the division time t_D^+ as clarified earlier (e.g. Hong [2]). For exponential growth (i.e. constant μ^+ and μ^-) with constant p, these parameters may be expressed as

$$t_D^+ = (\ln 2)/\mu^+ \tag{11}$$

and

$$t_d^+ = (\ln 2)/[(1-p)\mu^+] \tag{12}$$

Accordingly, τ may be defined either in terms of t_D^+ or t_d^+.

Thus, if τ is defined as

$$\tau = t/t_D^+ \tag{13}$$

then one may obtain from Eq. (9),

$$X^+/X_0^+ = (2-\beta)^{\tau} \tag{14}$$

where, β, the segregation coefficient, is related to p by

$$(1-p)\ln 2 = \ln(2-\beta) \tag{15}$$

On the other hand, if τ is defined as

$$\tau = t/t_d^+ \tag{16}$$

then instead of Eq. (14) one must use the equation

$$X^+/X_0^+ = 2^{\tau} \tag{17}$$

to describe the system dynamics.

Thus, depending on whether the term generation refers to a generation of cell division or a generation of cell growth (i.e. doubling), the definition of τ shall be given by Eqs. (13) or (16) respectively, and the fundamental equation describing the dynamics of the P^+ population will accordingly refer to Eqs. (14) or (17). If, however, either of μ^+ or p or both are not constant, then Eqs. (13) and (16) must be modified adequately, as discussed below, in order to define τ for these situations.

Eq. (14) is derived on the implicit assumption that β (hence p) is constant. Thus, when the latter is a variable, Eq. (17) should be used instead to describe the system dynamics. It follows consequently from Eq. (9) that τ must then be defined as

$$\tau = \left(\int_0^t (1-p)\mu^+ \, dt\right) \Big/ \ln 2 \tag{18}$$

The situation when only μ^+, but not p, is variable may also be considered. Here, Eq. (14) may be used, so the definition of τ takes the form

$$\tau = \left(\int_0^t (1-p)\mu^+ \, dt\right) \Big/ \ln(2-\beta) \tag{19}$$

which, on simplification using Eq. (15), becomes

$$\tau = \left(\int_0^t \mu^+ \, dt\right) \Big/ \ln 2 \tag{20}$$

It may be easily verified that for constant μ^+, Eq. (13) follows from Eq. (20). Thus, for constant β and variable μ^+, Eq. (14) still holds. For this case, therefore, the general definition of τ is given by Eq. (20).

Assuming the above definition to be valid, the growth dynamics of the P^- population in the τ-domain may now be analyzed. The basis of this analysis is similar to that used by Srienc et al. [3] for evaluating the time course of growth of P^- cells following a segregated approach in the real time domain. Consequently, the concentration of P^- cells in the culture at the instant τ, is viewed conceptually and mathe-

matically as the sum over all z, $(0<z<\tau)$ of the concentrations of P^- cells, at τ, that originated from P^+ cells at the instant z, and which have been growing for an interval $(\tau-z)$.

Considering the situation when $\chi_0=0$ (where $\chi=X^-/X^+$ and subscript "0" indicates the initial instant), i.e. when no P^- cells are present initially, it follows from Eq. (10) that the instantaneous rate of formation of P^- cells (from P^+ cells) at time "t" in the real time domain is given by

$$dX_t^-/dt=p\mu^+X_t^+ \tag{21}$$

If z denotes the corresponding instant in the τ-domain, then, using Eqs. (14) and (20), the above equation may be expressed in the τ-domain as:

$$dX_z^-/dz=\left[\ln\left(\frac{2}{2-\beta}\right)\right]X_0^+(2-\beta)^z \tag{22}$$

It may be noted here that X_t^- and X_z^- denote the same quantity.

The "history parameter", $\{\theta\}_z^\tau$ for P^- cells may now be introduced. If the subpopulation of P^- cells originating at the instant z be in their ith generation at the current instant τ then

$$(i-1)=I[\{\theta\}_z^\tau] \tag{23}$$

i.e. with reference to Eq. (8) it may be said that $\{\theta\}_z^\tau$ is the analogue of τ for P^- cells. Further, in analogy with Eq. (7), it may be inferred that the concentration of the said subpopulation increases by a factor $2^{\{\theta\}_z^\tau}$ with respect to its value at the instant "z". This may be expressed mathematically as

$$dX_{z,\tau}^-=2^{\{\theta\}_z^\tau}dX_z^- \tag{24}$$

Combining Eqs. (22) and (24), we finally arrive at an expression for the overall concentration of P^- cells, $\{X^-\}_0^\tau$ at the instant τ, for the condition $\chi_0=0$, i.e.

$$[\{X^-\}_0^\tau/X_0^+]_{\chi_0=0}=\ln\left(\frac{2}{2-\beta}\right)\int_{z=0}^{z=\tau}2^{\{\theta\}_z^\tau}(2-\beta)^z\,dz \tag{25}$$

If, instead of the overall concentration, we require the concentration, $\{X^-\}_{z_1}^\tau$, say, where $0<z_1<\tau$ then this quantity may be obtained by simply changing the lower limit of integration in Eq. (25) from $z=0$ to $z=z_1$.

It may be shown that the initial presence of P^- cells in the system (i.e. $\chi_0>0$) mathematically translates to the addition of a separate term to the quantity $[\{X^-\}_0^\tau/X_0^+]_{\chi_0=0}$ as shown below:

$$[\{X^-\}_0^\tau/X_0^+]_{\chi_0>0}=[\{X^-\}_0^\tau/X_0^+]_{\chi_0=0}+\chi_0 2^{\{\theta\}_0^\tau} \tag{26}$$

An expression for evaluation of the "history parameter", $\{\theta\}_z^\tau$ is still required. If the ratio of the specific growth rates, α, where α is defined as

$$\alpha=\mu^-/\mu^+ \tag{27}$$

is constant, then, following earlier investigators (e.g. Park et al. [4]) $\{\theta\}_z^\tau$ may be expressed as

$$\{\theta\}_z^\tau=\alpha(\tau-z) \tag{28}$$

In general, when α is variable, $\{\theta\}_z^\tau$ is given by

$$\{\theta\}_z^\tau=\int_z^\tau\alpha(z)\,dz \tag{29}$$

the notation $\alpha(z)$ signifying that α is a function of the dimensionless time variable z. For constant α, the result expressed in Eq. (28) follows directly from Eq. (29). If the situation under consideration permits the use of a constant α (e.g. if the specific growth rates are Monod functions of a limiting substrate, then the relative values of the saturation coefficients for the P^+ and P^- strains determine the value of α, as discussed earlier by the present authors [5]), then using the corresponding value of $\{\theta\}_z^\tau$ from Eq. (28), the integration in Eq. (25) may be performed analytically. In general, if z_1 and z_2 be the lower and upper limits of integration, then the integral, I, (say) in Eq. (25) may be expressed as:

$$I=\int_{z_1}^{z_2}2^{\alpha(z_2-z)}(2-\beta)^z\,dz \tag{30}$$

for which the integrated result is

$$I(2^{\alpha z_2}/\ln\phi)(\phi^{z_2}-\phi^{z_1}) \tag{31}$$

where

$$\phi=(2-\beta)/2^\alpha \tag{32}$$

For $z_1=0$ and $z_2=\tau$, using the result expressed in Eq. (31), one obtains from Eq. (25).

$$[\{X^-\}_0^\tau/X_0^+]_{\chi_0=0}=\ln\left(\frac{2}{2-\beta}\right)\frac{2^{\alpha\tau}}{\ln\phi}(\phi^\tau-1) \tag{33}$$

One of the primary objectives of τ-domain analysis in a recombinant culture is to use Eq. (25) to obtain segregated information on generation specific subpopulations of the P^- cell population. Nevertheless, the result expressed in Eq. (33) serves to certify the validity and correctness of the τ-domain analysis. If the t-domain Eqs. (9) and (10) are solved for constant p and α, with $\chi_0=0$, and the expression thus obtained for (X^-/X_0^+) be transformed to the τ-domain, the final result is identical to Eq. (33).

Suppose it is required to find the fractional concentration of those P^- cells in a recombinant culture that are growing for $\leq k$ generations at real time t_1. If z_k denotes the instant (in the τ-domain) when this subpopulation first appeared and τ denotes the current instant (corresponding to real time t_1), then, in the general case, z_k may be evaluated from Eq. (29) using $z=z_k$ and $\theta_{z_k}^\tau=k$, i.e.

$$k=\int_{z_k}^\tau\alpha(z)\,dz \tag{34}$$

Assuming that $\alpha(z)$ values may be obtained against corresponding z values, the above equation may be solved for z_k by trial, after replacing the integral by an appropriate quad-

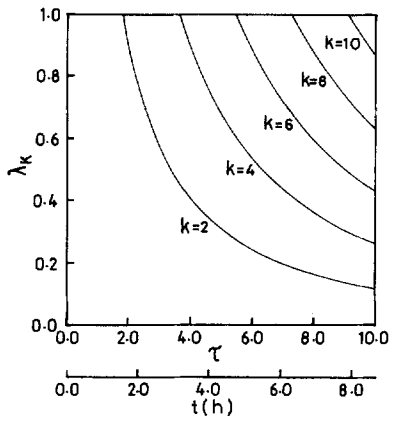

Fig. 1. Progress of the fractional concentration λ_k with the number of generations (τ) and real time t (in hours) for different "history parameters".

rature formula. Next, denoting the fractional concentration of the subpopulation under consideration (expressed as a fraction of the overall P^- population) by λ_k, the latter may be evaluated using the following expression:

$$\lambda_k = \frac{\int_{z_k}^{\tau} 2^{\{\theta\}_z^{\tau}}(2-\beta)^z \, dz}{\int_0^{\tau} 2^{\{\theta\}_z^{\tau}}(2-\beta)^z \, dz} \tag{35}$$

For constant α, the above expression simplifies to

$$\lambda_k = \frac{\phi^{\tau} - \phi^{z_k}}{\phi^{\tau} - 1} \tag{36}$$

The fraction ρ_{i_L,i_U}, of P^- cells that have a "history parameter" i^- where $i_L \le i^- \le i_U$ may be calculated directly using the parameter λ, as shown below

$$\rho_{i_L,i_U} = \lambda_{i_U} - \lambda_{i_L} \tag{37}$$

The usefulness of τ-domain analysis may be illustrated by showing in graphical form the progress of the fractional concentration λ_k with the number of generations for different "history parameters", i.e. for different values of k. Towards this end, 10 generations of exponential growth in a recombinant culture with the kinetic parameters, say, $\mu^+ = 0.80\ h^{-1}$; $\mu^- = 0.88\ h^{-1}$; $\beta = 0.05$; has been considered as an example. From the stated conditions it follows that α is constant, so that z_k may be obtained from the relation

$$k = \alpha(\tau - z_k) \tag{38}$$

which follows from Eq. (34) for constant α; and Eq. (36) may be used for calculating λ_k. Further, since μ^+ is constant, Eq. (20) reduces to the form

$$\tau = \mu^+ t/(\ln 2) \tag{39}$$

which may be used to obtain values of real time t (h) corresponding to τ. In the present example, λ_k vs. τ plots have been constructed for five different values of k, viz. $k = 2, 4, 6, 8, 10$; and these are shown in Fig. 1. The real time scale corresponding to the dimensionless time scale has also been given in the figure. Evidently, Fig. 1 may also be used to calculate the parameter ρ_{i_L,i_U} as defined by Eq. (37).

3. Conclusions

The above analysis shows the usefulness of following a dimensionless time domain approach in investigating the growth dynamics of recombinant cultures in situations where the emphasis is on obtaining quantitative information on the population dynamics in terms of generations. For recombinant cell systems where the culture concentration is a function of the cell histories, quantitated in terms of generations, e.g. cultures growing under auxotrophic selection pressure (Satyagal and Agrawal [6]), the τ-domain analysis that has been developed in this paper is expected to be particularly useful. In such systems, the number of generations of cell growth that may be sustained by a newly originating P^- cell, in batch culture, depends on the amount of the essential nutrient originally inherited by the cell during its formation from a P^+ cell through plasmid segregation. Thus, the overall concentration of P^- cells in the culture becomes a function of the distribution of individual cell histories quantified in terms of generation units. The use of τ-domain approach in such situations seems to be the preferred alternative to a real time analysis.

4. Nomenclature

i, i^-	integral generation number of plasmidless cells
I	integral occurring in Eqs. (25) and (30)
j	integral generation number "for a culture in the jth generation"
k	a particular value of the "history parameter" $\{\theta\}_z^{\tau}$ for $z = z_k$
p	relative segregation rate of plasmid-bearing cells
P^+	plasmid-bearing cells
P^-	plasmidless cells
t	real time (h)
t_d	doubling time of cells (h)
t_d^+	doubling time of plasmid-bearing cells (h)
t_D^+	division time of plasmid-bearing cells (h)
X	concentration of cells in culture (in appropriate units)
X^+	concentration of plasmid-bearing cells
X^-	concentration of plasmidless cells
z	dummy variable of integration in the τ-domain; also, an arbitrary instant in the τ-domain
z_1, z_2	particular values of z
z_k	value of z corresponding to the value $\{\theta\}_z^{\tau} = k$

Greek symbols

α	ratio of specific growth rates of plasmidless and plasmid-bearing cells ($= \mu^-/\mu^+$)

β segregation coefficient of plasmid-bearing cells
χ ratio of concentrations of plasmidless to plasmid-bearing cells ($=X^-/X^+$)
χ_0 initial value of parameter χ (i.e. at $t=0$)
ϕ parameter defined by Eq. (32)
λ_k fractional concentration of plasmidless cells with "history parameter" $\leq k$, expressed as a fraction of the overall concentration of plasmidless cells
μ specific growth rate of cells (h^{-1})
μ^+ specific growth rate of plasmid-bearing cells (h^{-1})
μ^- specific growth rate of plasmidless cells (h^{-1})
ρ_{i_L,i_U} fractional concentration of plasmidless cells having a "history parameter" i^- given by $[i_L \leq i^- \leq i_U]$
$\{\theta\}_z^\tau$ "history parameter" of plasmidless cells originating at the instant z in the τ-domain, at the current instant τ
τ dimensionless time variable denoting the number of generations, fractional or integral, in a culture; also denotes the current instant in the dimensionless time domain

Superscripts

$+$ plasmid-bearing cells
$-$ plasmidless cells

Subscripts

k corresponding to the value of "history parameter" $=k$
L lower limit
0 initial instant (i.e. $t=0$ or $\tau=0$)
t the real time instant, t
τ the dimensionless time domain instant, τ
U upper limit
z the instant z in the dimensionless time domain

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